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Evaluation Solvent Level Effect on Sugar Yield during Microwave-Assisted Pretreatment

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Abstract. An efficient pretreatment step is essential to ensure maximum and economical recovery of the fermentable sugar from enzymatic hydrolysis of lignocellulosic biomass. Microwave-assisted pretreatment was found to be a good alternative of conventional heating pretreatment of acid or alkali due to its direct heating of sample which reduce the energy consumption. The present study aims to evaluate the effect of using very diluted solvent (acid and alkali) on monomeric sugar yield during microwave-assisted pretreatment of lignocellulosic biomass (sago palm bark wastes). The changes of the surface structure of untreated and pretreated lignocellulosic biomass were detected using SEM analysis. Three solvents were employed at three concentrations (0.01, 0.05 and 0.1 N). The solvents that utilized in the microwave-assisted pretreatment were sulphuric acid (MA), sodium hydroxide (MH) and sodium bicarbonate (MB) and compared with distilled water (MW). Sago palm bark (SPB) was soaked in these solvents as a substrate at 5% w/v and subjected to microwave power at 800 W in a modified household microwave oven for 5 minutes. Immersing feedstock in distilled water and heating using microwave heating released 5.32 mg/g of monomeric sugar. Highest yield of monomeric sugar was found using 0.1N H_2SO_4 which yielded 10.28 mg/g during. Applying the pretreatment using the similar concentrations of NaOH and $NaHCO_3$ released 9.35 and 4.31 mg/g of monomeric sugar, respectively. Decreasing chemical solvent level from 0.1 to 0.05 and 0.01 N shows a negative influence on sugar yield for MA and MH pretreatment while MB pretreatment displays a positive effect by extracting some useful components such as xylose.

Keywords: Microwave- Assisted pretreatment, Sugar, Solvent level. Lignocellulosic Biomass

1. Introduction

In recent years, the global concern about the food security, energy sources, and the environmental issues have increased as a result to the dramatic increasing in population growth an food prices, depleting of raw fossil materials and worldwide climate change [1-2]. Lignocellulose is one of the most economically and environmental friendly alternatives to produce liquid fuels such as biofuels and bioethanol [3]. Lignocellulosic materials comprises of wastes from agricultural residues. These materials can be considered as an attractive source for sugar alcohol production due to lower cost, available at large quantities and do not cause food shortage [4]. In addition, recycling the biomass wastes supports effective the solid waste management systems [5-9]. Sago palm bark is one of a by-product of agriculture industries. In Malaysia, the production of sago starch process includes using sago palm trunk as the main source to extract the starch and disposes the sago palm trunk bark (cortex) as a waste of sago starch industry [10].

Lignocellulosic biomass comprises of three major components; and lignin in addition to other minor components namely ash pectin, protein and extractives. Both cellulose and hemicellulose comprise of polymeric sugars which create the potential to release fermentable sugars such as glucose and xylose during the hydrolysis stage which in turn, can be utilized in the manufacture of other products. The pretreatment process is essential for bioconversion of lignocellulose. It facilitates the hydrolysis process of polymeric sugars and increase fermentable sugars yield. Various technologies have applied for pretreatment methods such as hydrothermal treatment [11], wet oxidation [12], and steam explosion [13] have been investigated; however, these methods have some negative aspects to implement such as very high pressures and temperature. Therefore, there is a global trend to focus on

the use of technologies that utilize green chemical transformation into value-added derivatives. Thus, the minimal use of energy and auxiliaries requirements e.g. microwave heating applications and diluted solvents will provide viable and sustainable routes for the manufacturing of commodities specifically in nutraceutical and biorefinery industries.

Microwave-assisted pretreatment has gained more acceptance due to high efficiency and easy operation. Recent studies on microwave-assisted pretreatment have been conducted and have demonstrated that microwaves capability to change the ultrastructure of lignocellulosic biomass [14,15], break down the recalcitrant bond among lignin, cellulose and hemicellulose and enhance releasing the corresponding monomers (fermentable sugars)sugar recovery during enzymatic hydrolysis. The use of water was reported as a first solvent that applied during microwave-assisted pretreatment which has positive reflection on the enzymatic hydrolysis of lignocellulosic dry matter [16]. Microwave heating can be easily combined with chemical solvents and accelerate the chemical reaction rate [17]. Therefore, utilization of 'green' solvents is encompassed by the overall goal to minimize the resulting environmental impact from the use of solvents in chemical production. Combination of microwave heating with dilute solvents during pretreatment could aid to cost-effective pretreatment method as it can reduce energy usage, degradation of product and decrease corrosion problems associated with concentrated solvents [18].

The objective of this study is to investigate the minimal solvent level that is capable to achieve high monomeric sugar yield from microwave pretreatment liquor of lignocellulosic bioamss. Three types of diluted solvents in low concentrations were employed to perform microwave-assisted pretreatment including sulfuric acid, sodium hydroxide and sodium bicarbonate and compared to distilled water as a control. The effect of concentration of solvent on solid loss percentage and pH of liquid phase prior and after microwave treatment were also reported. The morphology analysis of untreated and pretreated biomass was evaluated to investigate the structural differences affected during microwave pretreatment process.

2. Material and methods

2.1 Feedstock

In this study sago palm bark waste is used as lignocellulosic biomass. The sago palm trunk logs were purchased from a local plantation in Melaka, Malaysia. Preparing of biomass was included debarking these logs to separate the outer part (bark) from inner part (pith) as shown in Figure 1.The collected bark was firstly dried at105 ºC for 24 h until constant weight. Then the dried bark chipped by wood chipper and sieved using chips classifier machine to obtain particle size of 2-3 cm and finally stored in plastic bags at -20 ºC for further experiments.

Figure 1. Preparation steps of sago palm bark

2.2 Microwave pretreatment
A household microwave oven with frequency of 2.45 GHz (Panasonic, NN-ST340M) was modified to perform microwave-assisted pretreatment. SPB samples were loaded at 5% (w/v) into 1-L round-bottom flask connected to reflux condenser through 25 mm round hole at the centre of microwave cavity and all samples were subjected to 800 W microwave powers for 5 minutes. The chemical solvents of pretreatment were prepared by diluting sulphuric acid, sodium hydroxide, and sodium bicarbonate (R&M Chemical, Malaysia) using deionized water to obtain 0.01, 0.05 and 0.1 N concentrations of these chemicals. After pretreatment, the mixture was filtered through a filter paper (Double Ring filter paper 102, China) to separate solid residue and liquid (hydrolsate). The filtered solid fraction were washed with deionized water and dried at 60 ℃ for until constant weight and then weighted to calculate the solid loss percentage. The liquid fraction of pretreated biomass was collected and filtered for HPLC analysis for sugar content.

2.3 Analytical procedure
A high-performance liquid chromatography (HPLC) system (Alltech ELSD 2000, ALT, USA) equipped with a RI detector and Rezex RPM-Monosaccharide Pb+2 columns (Phenomenex Inc., USA) using mobile phase deionized water was used to analyse monomeric sugars. The monomeric sugar standards of D-(+)-Glucose, and D-(+)-xylose (Sigma Aldrich, USA) were used for sugar quantitative analysis according to the Renewable Energy Laboratory (NREL) procedure [19]. The morphology of untreated and pretreated biomass were carried out using scanning electron microscope (SEM) (S-3400N, Hitachi, Japan) at various magnifications and observed using a voltage of 5-10 kV to detect the surface structure changes.

3. Results and Discussion

3. 1 Effect of solvent on solid loss
The solid loss in the weight of pretreated biomass is an indicator of the destruction plant cell during the pretreatment process.The percentage solid loss of the different microwave-assisted solvents pretreatment were calculated and depicted in Figure. 2. It can be obviously observed that increasing the concentration of NaOH (MH) led to increase the solid loss significantly. This might be attributed to the fact that is believed that a lignocellulose component lignin is highly NaOH-soluble [16]. While, using different concentrations of H_2SO_4 (MA), do not show big significant differences. They showed only slightly better than using distilled water (9.35%). It was proposed that acid pretreatment has the ability to remove the hemicellulose fraction but has less ability to remove lignin that tighten hemicellulose and cellulose in lignocellulosic materials [20] thus it caused a low solid loss. Highest solid residue loss was 16.6% when using 0.1 N NaOH solution. For MB pretreatment, the increasing in concentration of $NaHCO_3$ resulted in a reduction of solid loss percentage. This trend might be caused by the chemical behavior of sodium bicarbonate in the aqueous solutions. The aqueous solutions of $NaHCO_3$ are mildly alkaline because of formation of carbonic acid and hydroxide ion. Increasing the concentration of sodium bicarbonate will also increase the formation carbonic acid thus decreasing of alkalinity effect and neutralize the solution. It was reported, the pretreatment with presence of alkaline solution decreases the solid residue content because the solid loss came from the solubility of the components such as lignin and hemicelluloses in alkaline solution in microwave pretreatment [21]. Generally, the combination of microwave heating and chemical pretreatment has positive effect in destroying the recalcitrant structure of lignocellulose because the acidic solution removes hemicellulose while alkaline solution removes lignin.
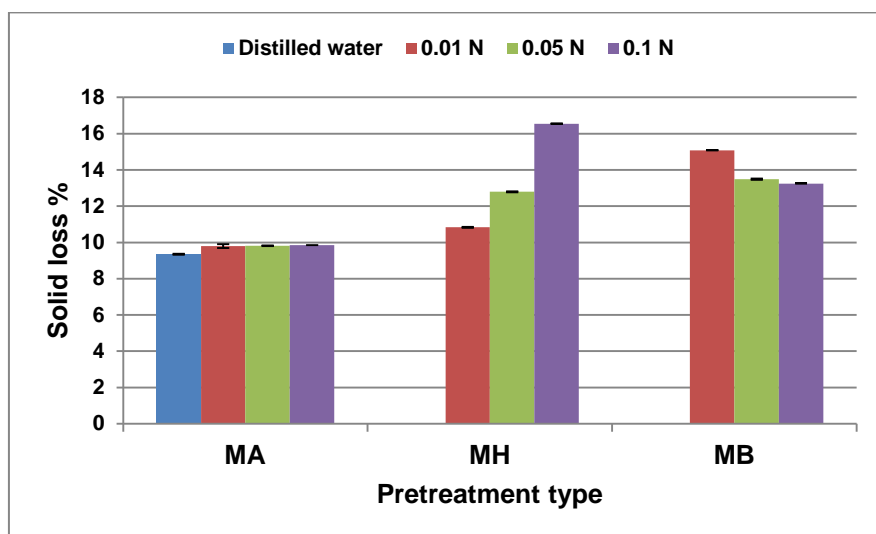
Figure 2. Solid loss percentage at different solvent levels

3.2 Effect of pretreatment solvent on hydrolysate pH

The pH values of pretreatment solutions prior and after microwave-assisted pretreatment are shown in Table 1. When distilled water was used as the solvent (MDW) the pH value of the solution dropped significantly from 6.95 and 4.28. This could be related to the formation of organic acids such as acetic acid, formic acid and lactic acid [22], from the pretreated matters degraded during the pretreatment process. Sulphuric acid as solvent at all concentrations only decrease slightly after the microwave-assisted pretreatment. The pH values of microwave-sodium hydroxide pretreatment shows same trend of declining in pH values after pretreatment of SPB at 0.05 and 0.1 N. However, the pH value of treatment using 0.01 N of NaOH dropped more significantly from 12.25 to 10.22 compared to 0.05 and 0.1 N NaOH. In contrast, in MB pretreatment the pH values increased after the pretreatment process. Several reasons might be interoperated presence of the fluctuation of pH values during combination of microwave heating and chemicals as a pretreatment rout. It was recorded that the hydroxide ions attacks the lignin and results in splitting ether bonds in the delignification process during the alkaline pretreatment, [23]. Moreover, the severe conditions such as high temperature that rises as a result to the high heating rate of microwave heating due may lead to form the sugar degradation products such as acetic acid and etc., which can cause the lowering of pH values [24]. As the increasing of temperature due to high microwave power, the acid proton performed as a catalyst to protonate the oxygen atom linkage between hemicellulose and cellulose. It is reported that the high temperature can contributes to the alkaline degradation of lignocellulosic biomass via cleavage the ester bonds among of biomass among the main components cellulose and hemicellulose and lignin. As well as, more intense alkaline biomass degradation throughout the intensive alkaline environment will result in more acidic substances such as acetic acid and uronic acid substitutions due to the hydrolyzing the acetyl groups (one of the major hemicellulose constituent) [25]. On contrary, it is of interest to observe that the pH values of the pretreated solution became a higher after $NaHCO_3$ pretreatment. This is might be because the pretreated biomass generally possesses buffering capacity, which can prevent acid formation and makes the pretreatment process conditions has less severity [26]. To sum all, the higher initial H_2SO_4 or NaOH concentrations results in lower final pH values of the pretreatment solution. This implies that the increase in acid or alkaline concentrations in microwave-assisted pretreatment is conducive to the destruction of the biomass even though the buffering capacity is exhibited by the biomass [27].

Table 1. pH values prior and after MW, MA, MH, and MB pretreatment

Pretreatment	Solvent concentration (N)	pH before pretreatment	pH after pretreatment
MW	NA	6.95	4.28 ± 0.007
MA	0.1	2.21	2.14 ± 0.056
	0.05	1.71	1.45 ± 0.155
	0.01	1.22	1.02 ± 0.355
MH	0.1	13.06	12.69 ± 0.141
	0.05	12.69	12.18 ± 0.084
	0.01	12.25	10.22 ± 0.396
MB	0.1	8.54	9.87 ± 0.31
	0.05	8.65	10.07 ± 0.148
	0.01	8.83	9.99 ± 0.091

3.3 Effect of solvent on sugar yield

The present study is investigating the dilute solvent to be used as combination with microwave heating to perform pretreatment process. The extracted monomeric sugars from SPB with MW, MA, MH and MB pretreatment are shown in Table 2. Only a total of 5.32 mg/g monomeric sugars was detected for the case that when using distilled water as the solvent. Highest value of total sugar yield was obtained at 10.28 mg/g using 0.1 N H_2SO_4. Meanwhile, performing microwave-alkaline pretreatment using 0.1 N NaOH and 0.1 N $NaHCO_3$ at the same condition resulted less amounts of monomeric sugar at 9.35 mg/g and 4.31mg/g, respectively. However, the highest amount of glucose was found in MH pretreatment. This could be due the ability of alkaline solution to produce sugar by the reduction of degree of polymerization and crystallinity of cellulose thus enhancing solubilisation lignin fraction [28]. The presence of xylose fraction of monomeric sugar in the acidic pretreatment suggests dilute acid is enough to hydrolyse the hemicellulose part its respective monosaccharaides [29,30]. The less alkaline solution, 0.1 N $NaHCO_3$, produces less sugar compared to the MH pretreatment. It might be this solution could not disturb the recalcitrant structure of lignocellulose material where the pH near neutral.

　Reducing the solvents to 0.05 N show a decline trend of sugar yield except for MB. However, microwave-assisted pretreatment of the biomass particles using 0.01 N $NaHCO_3$ shows slight increment of sugar yield to 6.74 mg/g, while MA and MH pretreatment resulted in sugar yield of 4.52 and 3.68 mg/g, respectively. These results revealed that the combination of microwave heating and $NaHCO_3$ can release more monomeric sugar and able to extract different types of monomeric sugar such as xylose and arabinose compared with other type of chemicals at low concentration. This is refer to that the weak base of $NaHCO_3$ can partially hydrolysed the hemicellulose fraction due to its amphoteric behaviour, even though previous work reported that the hemicellulose fraction need strong alkaline conditions for hydrolysis [29, 31]. Moreover, it is shown that performing microwave-assisted pretreatment with 0.01 N H_2SO_4 resulted in sugar yield less than the one treated with distilled water. As whole, the synergistic of the solvents with microwave irradiation can enhance the release of the fermentable sugar with some limitations. One of these limitations is the solvent concentration. Thus, it is important to select the most suitable solvent concentration which can lead to an effective pretreatment process and enhance the following hydrolysis step outcome.

Table 2. Monomeric sugar yield after microwave-assisted pretreatment of biomass

Pretreatment type	Solvent	Solvent concentration (N)	Glucose mg/g	Xylose mg/g	Arabinose mg/g	Total mg/g
MW	**H_2O**	0	5.32	ND[*]	ND	5.32
MA	**H_2SO_4**	0.1	7.61	2.67	ND	10.28
		0.05	5.66	ND	1.13	6.79
		0.01	4.52	ND	ND	4.52
MH	**NaOH**	0.1	9.35	ND	ND	9. 35
		0.05	5.95	ND	ND	5.95
		0.01	4.26	ND	1.42	5.68
MB	**$NaHCO_3$**	0.1	4.31	ND	ND	4.31
		0.05	4.52	ND	0.37	4.52
		0.01	4.45	1.34	0.95	6.74

* ND=Not detected

3.4 Effect of solvent on morphological characteristics of biomass
The structural changes in the surface appearance of prior and after microwave-assisted pretreatment of biomass were detected using the scanning electron microscopy (SEM) and depicted in Figure 3. The general observations of untreated and pretreated biomass refers to that the microwave-assisted pretreatment induced physical changes in the structure biomass. It is easy to observe that the untreated biomass has compact and no pores present in the biomass surface as can be seen in Figure. 3A. meanwhile, the pretreated biomass has disturbed and fragment surface structure as shown in Figure.3 B, C, D and E. By the same token, the MW pretreated biomass exhibited some pores and rough surface in some parts (Figure. 3B). An obvious fragmentation of biomass surface structure was caused by MA, MH and MB due to microwave irradiation and acidic/alkaline environment and this is particularly noticeable when some fragments have flaked off from the lignocellulose surface. This indicates that the pretreatment procedure partially remove the external fibers and the rough surface generated from the pretreatment which in turn increase surface area so that enzymes will become more accessible during the hydrolysis stage causing the hydrolysis step to be more effective. A similar structural change were earlier recorded for pretreated corncob using the combination of sodium hydroxide and microwave heating [32] and for sugarcane bagasse was soaked with alkaline, or acid in microwave environment [20].
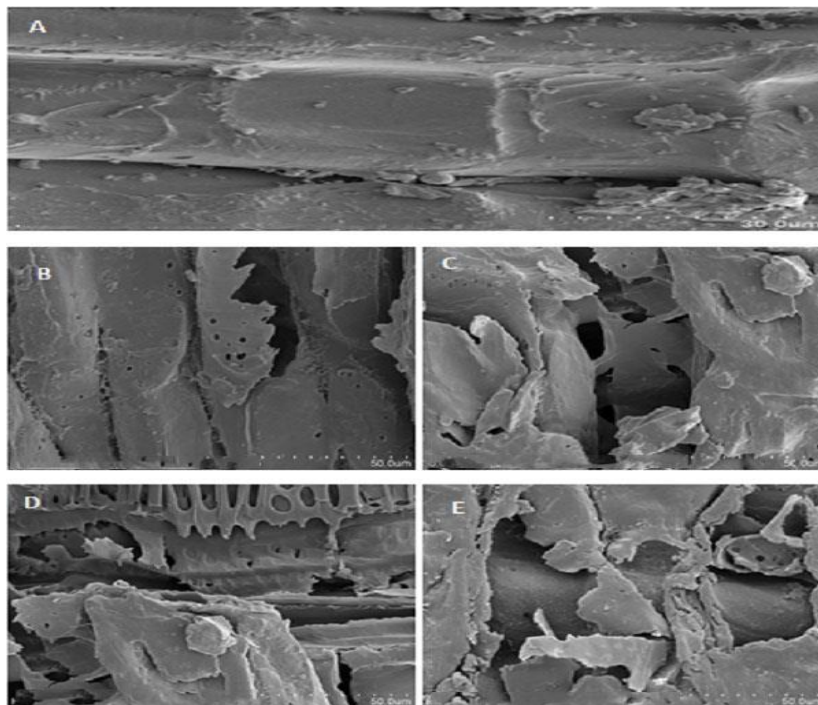
Figure 3. SEM images (A) untreated X 1500, (B) MW, (C)MA, (D) MH, and (E) MB pretreatment of biomass at magnification of X 1000 respectively.

4. Conclusion

The solvents were used during microwave-assisted pretreatment of biomass show that type of solvent and its concentration are important factor for the reducing sugar release. Higher concentration of H_2SO_4 and NaOH resulted in substantially improved monomeric sugar release during microwave-assisted pretreatment compared to water with maximum yield 10.28 mg/g of total sugar when using 0.1 N H_2SO_4 comprising of 7.61 mg/g glucose and 2.27 mg/g xylose. However, the highest amount of 9.35 mg/g glucose yield was found in microwave-assisted NaOH pretreatment at the same concentration. Therefore, from the operating point of view for sago palm bark wastes pretreatment, it is concluded that it can be considered is 0.1 N of H_2SO_4 is the more suitable solvent concentration to perform microwave pretreatment in terms the yield of different types of monomeric sugar such as xylose. Thus, using diluted acid solvent during microwave pretreatment may be will enhance subsequence enzymatic hydrolysis step due it is ability disturb cellulose and hemicellulose structure.

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